Green leaf volatiles disrupt and enhance response by the ambrosia beetle, *Gnathotrichus retusus* (Coleoptera: Scolytidae) to pheromone-baited traps

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ABSTRACT

Experiments were conducted to test the null hypothesis that green leaf volatiles, abundant in herbaceous plants and angiosperm trees, have no effect on the response by the conifer-infesting ambrosia beetle, *Gnathotrichus retusus* (LeConte), to pheromone-baited traps. A blend of four green leaf alcohols, 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol, each released at ca. 4 mg per 24 h, combined with a blend of two green leaf aldehydes, hexanal and (*E*)-2-hexenal, each released at ca. 13.0 mg per 24 h, reduced catches of females to levels not significantly different from those in unbaited control traps. Any of the four green leaf alcohols released alone disrupted responses of females, while 1-hexanol and (*E*)-2-hexen-1-ol strongly reduced catches of males. The two green leaf aldehydes released together, and (*E*)-2-hexenal released alone, weakly enhanced trap catches. These results lead to rejection of the null hypothesis on the basis of both positive and negative effects. Disruptive green leaf volatiles may have promise as forest product protectants against ambrosia beetles, by disguising hosts as non-hosts.

INTRODUCTION

Gnathotrichus retusus (LeConte) is one of three economically important ambrosia beetles in western Canada and the USA (Borden and McLean 1981). Together with *G. sulcatus* (LeConte) and the striped ambrosia beetle, *Trypodendron lineatum* (Olivier), *G. retusus* attacks green coniferous timber in the woods and in processing areas (Prebble and Graham 1957; Johnson 1958). The annual economic impact on the British Columbia (BC) coast was estimated by McLean (1985) to be $63 million (Can.), but this has since been updated to range from $95 to $189 million (Lindgren and Fraser 1994).

In timber processing areas in BC, ambrosia beetles have been the target of an integrated pest management (IPM) program since the early 1980's (Borden 1995). The primary components of the program are management of log inventories so as to minimize exposure of vulnerable logs to attack, and interception of host-seeking beetles by mass trapping them in semiochemical-baited traps. For *G. retusus* the attractive semiochemical baits are the aggregation pheromone (*S*)-(+)-6-methyl-5-hepten-2-ol (retusol) and the host tree kairomones ethanol and α-pinene (Borden *et al.* 1980a,b).

As effective as the IPM program is, the need remains for an efficient, cost-effective material that could be used to protect logs from attack. Such a material would disrupt response of beetles in some way to attractive pheromones and kairomones, e.g. through arresting or repelling them prior to their reaching the attractive source (Borden 1997). A

disruptant tactic would complement current management practices. Two potential repellents have been rigorously evaluated. Pine oil and oleic acid protected logs from attack by *G. sulcatus* and *T. lineatum* for 49.5 and 41.2 days, respectively (Nijholt 1980). However, both materials are relatively expensive, and pine oil is particularly unpleasant and difficult to work with. Another possible source of repellency is non-host volatiles such as green leaf volatiles (GLVs), six-carbon alcohols, aldehydes and derivative esters common to a wide variety of angiosperm trees and shrubs (Visser and Ave 1978; Visser 1986). GLVs have been demonstrated to have varying degrees of repellency to nine species of scolytid beetles (Dickens *et al.* 1992; Wilson *et al.* 1996; Borden *et al.* 1997; Savoie *et al.* 1998; Deglow and Borden 1998; Poland *et al.* 1998).

Borden *et al.* (1997) reported that, for *T. lineatum* in the BC interior, four green leaf alcohols [1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol] released alone or in a quaternary blend resulted in a 63% to 78% reduction of catches in traps baited with the aggregation pheromone, lineatin. In one of two experiments on the BC coast, the quaternary blend was weakly inhibitory. No inhibitory effect was found for the aldehydes, hexanal and (*E*)-2-hexenal, but in one of two experiments in the interior the binary blend caused a moderate enhancement of catches in lineatin-baited traps. Against *G. sulcatus*, only (*E*)-2-hexen-1-ol caused a significant reduction of catches in traps baited with the aggregation pheromone sulcatol (Deglow and Borden 1998). However, binary, ternary and quarternary blends of the above alcohols were all effective and caused disruption in an additive and redundant manner. Conversely (*E*)-2-hexenal alone and with hexanal weakly enhanced attraction.

Our objective was to test the null hypothesis that non-host GLVs (both aldehydes and alcohols) would have no effect on the aggregative response of *G. retusus* to its pheromone retusol.

MATERIALS AND METHODS

Experiments on *G. retusus* were set up in an abandoned dryland log sort at an elevation of 500 m, at North Bend, BC in the Interior Douglas-fir (IDF) biogeoclimatic zone (Hope *et al.* 1991). The forest is dominated by Douglas-fir, *Psuedotsuga menziesii* (Mirb.) Franco, with some black cottonwoods, *Populus trichocarpa* Torr. & Gray, paper birches, *Betula papyrifera* Marsh, and mixed deciduous brush near the edge. The sort was largely empty, except for some Douglas-fir and western red cedar logs, *Thuja plicata* Donn ex D. Don, stacked in the central area, and scattered piles of coarse woody debris. Twelve-unit multiple funnel traps (Lindgren 1983) were hung from ropes or poles, at least 15 m apart, and away from deciduous trees, around the perimeter of the dryland sort.

Three randomized complete block experiments (Exp.) were conducted, with dates and numbers of replicates as in Table 1, and chemical stimuli, sources, purities, release devices and release rates as in Table 2. In each experiment, pheromone-baited and unbaited control traps served as positive and negative control treatments, respectively, against which the bioactivity of GLV treatments added to retusol could be assessed. Exp. 1 tested an aldehyde blend, hexanal and (*E*)-2-hexenal, and an alcohol blend, 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol, alone and together. Exp. 2 tested the two aldehydes alone and together, and Exp. 3 tested the four alcohols alone and in a quaternary blend. Captured insects were stored frozen in plastic bags prior to sexing and counting.

Table 1

Numbers, dates, and numbers of replicates for field trapping experiments on *G. retusus* at North Bend, BC.

Exp. No.	Dates	Number of replicates[a]
1	8 May-4 June, 1996	♂5, ♀ 8
	24-29 June, 1996	♂ 7, ♀ 7
	29 June-6 July, 1996	♂2, ♀ 2
2	4-12 June, 1996	♂5, ♀ 7
	6-13 July, 1996	♂ 8, ♀ 10
3	13 July-30 Aug., 1996	♂6, ♀ 10

[a]Exp. 1-3 had 5, 5 and 7 treatments, respectively. A replicate represents all of the beetles captured in one randomized block of traps. In Exp. 1 and 2 treatments were re-randomized to produce new replicates on different dates. When no beetles of a given sex were captured in any trap within a replicate, that replicate was discarded, causing uneven numbers of replicates between sexes.

Table 2

Description of semiochemicals used in trapping experiments for the effect of GLVs on *G. retusus*.

Chemical[a]	Source[b]	Purity(%)[b]	Experiments	Release rate (mg per 24 h)[c]
retusol	P	100	1-3	5.0-6.0
hexanal	A	98	1, 2	13.0
(*E*)-2-hexenal	A	99	1, 2	13.0
1-hexanol	A	98	1, 3	3.8
(*E*)-2-hexen-1-ol	A	95	1, 3	3.8
(*Z*)-2-hexen-1-ol	B	92	1, 3	3.8
(*Z*)-3-hexen-1-ol	A	98	1, 3	3.8

[a]All GLVs stabilized with 1-2% (wet weight) Ethanox® 330 antioxidant, Ethyl Chemicals Group, Baton Rouge, LA

[b]P=Phero Tech Inc., Delta, BC; A=Aldrich Chemical Company, Milwaukee, WI; B=Bedoukian Research Inc., Danbury, CT. Purities as determined by manufacturer.

[c]All chemicals released from bubble caps (Phero Tech Inc.) at rates determined by Phero Tech in the laboratory at 22-24°C.

To satisfy criteria for normality and homoscedasticity, all data (except Exp. 1, males) were transformed by log(x+1) (Zar 1996). Means catches were compared by ANOVA (GLM procedure, SAS institute Inc. 1988) and the Ryan-Einot-Gabriel-Welsh Multiple Q-test (REGW test) (SAS Institute Inc. 1988; Day and Quinn 1989). For male *G. retusus* in Exp. 1, Friedman's nonparametric randomized block analysis of variance (Zar 1996) was used, as the data were non-normal and heteroscedastic. Values for missing data (four in Exp. 1 and two in Exp. 2) were estimated using Li's (1964) procedure (Zar 1996). In all cases α=0.05.

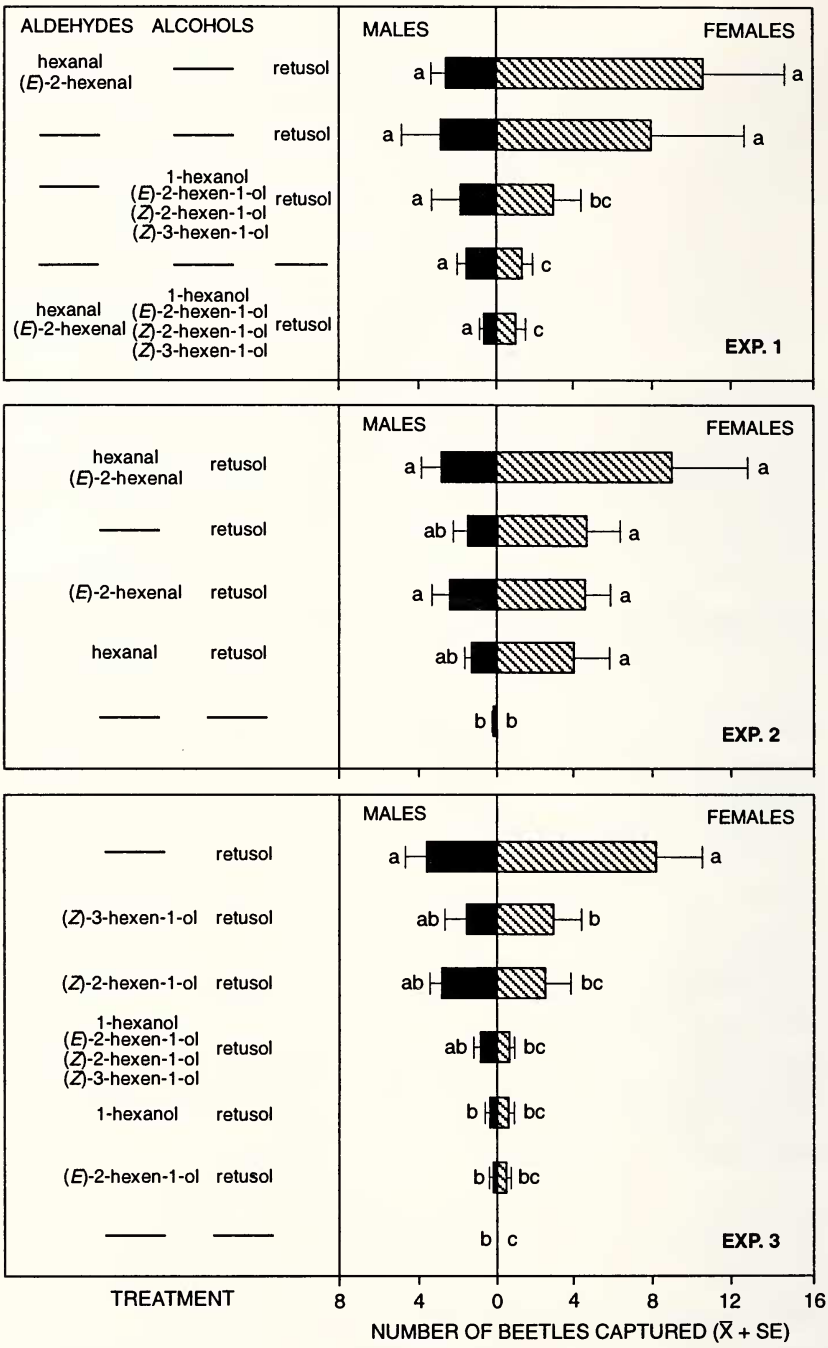

Figure 1. Captures (in rank order for females) in Exp. 1-3 of *G. retusus* to multiple-funnel traps baited with retusol alone or with a blend of two green leaf aldehydes and four alcohols (Exp. 1), the aldehydes alone and together (Exp. 2), and the alcohols alone and together (Exp. 3). Long dash indicates no treatment. In Exp. 1, bars for females with the same letter are not significantly different, REGW test, $P<0.05$, n=17; for males Friedman's nonparametric randomized block analysis of variance failed to detect significant differences, $P>0.05$, n=14. In Exp. 2 and 3, bars with the same letter are not significantly different, REGW test, $P<0.05$. For males and females in Exp. 2 and 3, respectively, n=13 and 17, and 6 and 10.

RESULTS

In Exp. 1, the aldehyde-alcohol blend reduced catches of female *G. retusus* in retusol-baited traps to levels not significantly different from those in unbaited control traps (Fig. 1). Males did not discriminate at all between treatments. In Exp. 2, males responded at levels significantly greater than to unbaited control traps when retusol was combined with the aldehyde blend or (*E*)-2-hexenal (Fig. 1). Females did not discriminate between retusol alone or with either or both green leaf aldehydes. In Exp. 3, both 1-hexanol and (*E*)-2-hexen-1-ol reduced responses by males to levels significantly lower than to retusol, and not different from those to unbaited control traps (Fig. 1). For females, all alcohol treatments caused catches to be significantly lower than to retusol alone, and all but (*Z*)-3-hexen-1-ol reduced catches to levels that could not be discriminated from those in unbaited control traps.

DISCUSSION

Our results demonstrate that green leaf volatiles can both enhance and disrupt the response of *G. retusus* to its aggregation pheromone. Therefore, the null hypothesis is rejected on the basis of both positive and negative effects. A similar disruptive effect was achieved with various GLVs on other conifer-inhabiting scolytids (Deglow and Borden 1998). However, enhancement of response to aggregation pheromones by GLVs is known to occur only in response to aldehydes by the ambrosia beetles *G. sulcatus* (Deglow and Borden 1998) and *T. lineatum* (Borden *et al.* 1997), and to 1-hexanol, a multifunctional pheromone for the bark beetle *Pityogenes knechteli* Swaine (Savoie *et al.* 1997). The weak attractive effect of hexanal and the aldehyde blend for *G. retusus* in Exp. 2 was overridden by the disruptive effect of the alcohols when the aldehyde and alcohol blends were combined in Exp. 1.

The powerful disruptive effect of the alcohols on females most likely reflects their strong response to pheromone in the absence of the alcohols. However, despite the moderate response by males to retusol, the disruptive effect of 1-hexanol and (*E*)-(2)-hexen-1-ol in Exp. 3 was still evident. It would be highly adaptive for pioneer male *G. retusus* to use any olfactory signal that would allow them to discriminate between potential hosts and non-hosts, thereby avoiding the risks of predation, desiccation, and metabolic expenditure associated with close-range inspection and rejection of non-hosts (Gries *et al.* 1989; Schroeder 1992).

By preventing host-seeking *G. retusus* from landing at or near attractive sources, disruptant green leaf alcohols offer considerable promise as log protectants, possibly in combination with attractant-baited traps if used in a push-pull treatment (Lindgren and Borden 1993). Although *G. sulcatus* is strongly repelled by the same green leaf alcohols that disrupt *G. retusus* (Deglow and Borden 1998), *T. lineatum* on the BC coast is not (Borden *et al.* 1997). Therefore other non-host volatiles, e.g. bark volatiles (Borden *et al.* 1998), might be needed in a formulation that would be equally effective on all three species of ambrosia beetles.

Further research is necessary to determine if attractive aldehydes or other compounds actually occur in attractive hosts, and if such compounds could be used to enhance the power of attractant-baited traps in IPM of ambrosia beetles.

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